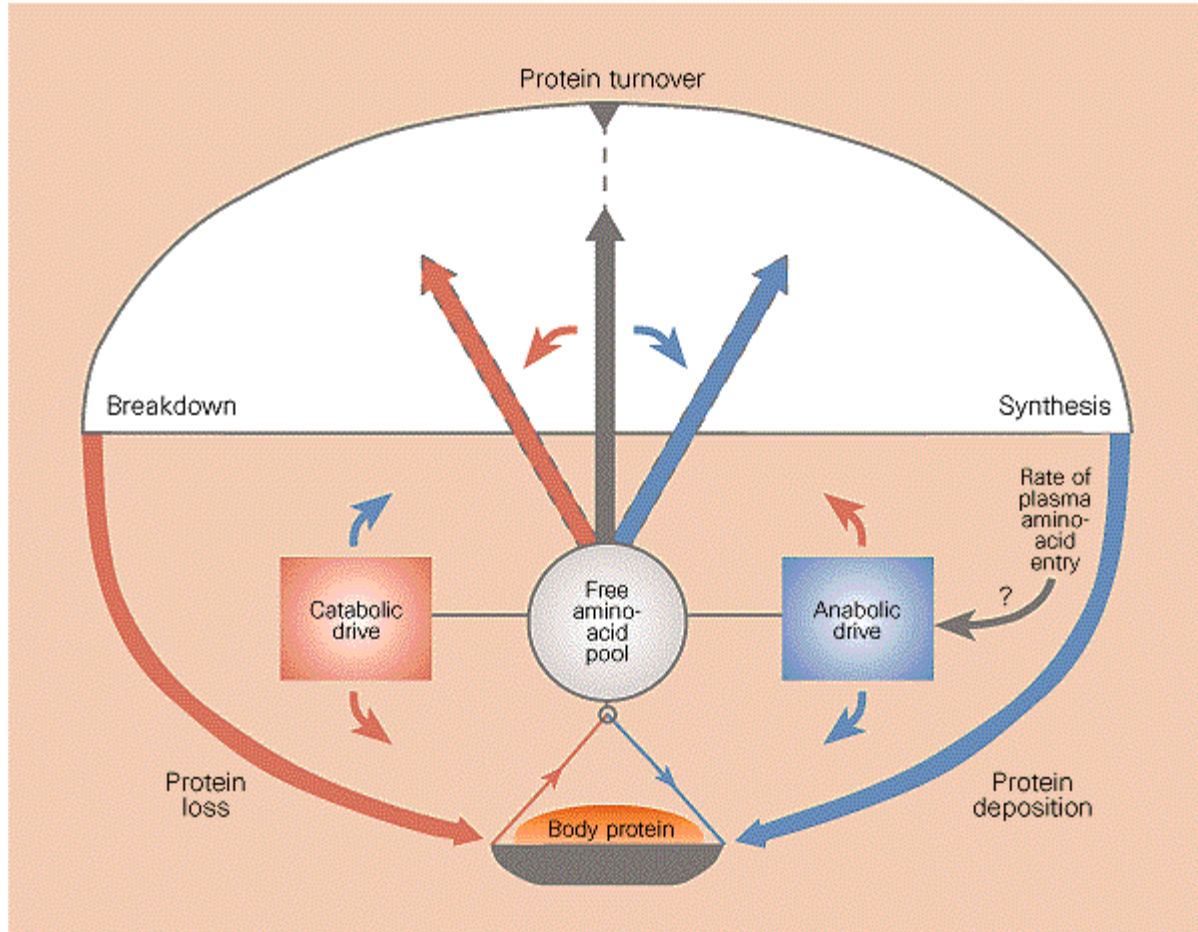




Protein Turnover Tracer Studies



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Determination of Whole Body Protein Turnover

Background	<p>It is necessary to employ tracer methods using isotopically-labeled compounds, to explore dynamic aspects of amino acid and protein metabolism in the whole organism. Various approaches have been followed in studies of human protein and amino acid metabolism. These approaches use a constant infusion of tracer that is coupled with the measurement of end products of N metabolism (Picou and Taylor-Roberts Model) or with the determination of the enrichment of isotope (^{15}N or ^{13}C) in plasma following administration of a labeled amino acid (Waterlow Model). When this latter approach is combined with measurements of ^{13}C in expired carbon dioxide or ^{15}N in urinary urea or ammonia, the components of whole body amino acid flux and whole body protein synthesis and catabolism can be determined.</p>
Picou Taylor-Roberts Method	<p>The Picou and Taylor-Roberts model was published in 1969 as a method to measure total protein synthesis and catabolism in infants. The method is still valuable today to compare whole body protein metabolism in different nutritional states, during sepsis and trauma, hormonal manipulation, and other metabolic conditions. The method is easy to employ in many applications with infants, surgical/trauma patients or outpatient volunteers.</p>
Route of Tracer	<p>The ^{15}N-glycine tracer can either be administered intravenously or orally continuously over a nine to 24 hour period. The tracer may be given as small oral bolus doses over the infusion period.</p>
Problems	<p>The Picou Taylor-Roberts Model will yield different results depending on the route of tracer administration, end-product measured (urea, ammonia, or total N), and time period of urine collected. Many studies have shown that these factors influence the final numbers. However, the method is useful for showing relative changes in whole body protein metabolism.</p>
Protocol	<p>Best Tracer: ^{15}N-glycine Priming Bolus Dose: 0.5 mg/kg (every 3 hours if tracer just given orally) Infusion Pump Speed: 0.174 cc/min Infusion Rate: 0.05 mg/kg/hr Infusion Time: 9 hours Sampling Times: 0, 9 hours urine collections Diet Protocol: Fasted or Fed References: Picou and Taylor Roberts, Clin. Sci. 36:283-296, 1969 Kien et al., Ann. Surg. 187:383-391, 1978 Kien et al., Metabolism 27:27-34, 1978</p>

Waterlow Model

Waterlow and Stephen in 1967 proposed an alternative approach to the Picou and Taylor-Roberts Model. The advantage of the Waterlow Model is that most studies can be completed within two hours as compared with the Picou and Taylor-Roberts Model which uses 9 hours or longer. In this model, a single labeled amino acid is infused continuously as a representative tracer for body protein amino acids. The dilution of this tracer in the blood and the catabolism to either carbon dioxide or urea is measured. The body protein turnover is calculated from an average amino acid composition of total body protein.

Components of the Waterlow Model

The basic tenets of the Waterlow Model describe amino acid flux as equal to the sum of all amino acid inflows to the plasma compartment. These would include dietary intake and inflow from protein breakdown. In the steady-state, the inflows to the plasma compartment will be equal to all outflows. These outflows would be the sum of amino acid oxidation and amino acid incorporated into protein synthesis. Thus, by determining amino acid flux, or isotopic dilution of tracer in the plasma, knowing dietary intake, and determining amino acid oxidation (catabolism), protein synthesis and protein breakdown are easily calculated as described below:

$$\begin{aligned} \text{Amino Acid Flux} &= \text{Amino Acid Oxidation} + \text{Amino Acid Incorporated into Protein} \\ &= \text{Amino Acid Dietary Intake} + \text{Amino Acid from Protein Breakdown} \end{aligned}$$

Amino Acid Tracers

The Waterlow Model has been used with several amino acid tracers, such as leucine, valine, threonine, proline, methionine, phenylalanine, tyrosine and lysine. We will outline a few representative approaches below.

Lysine as a Tracer

The determination of whole body protein turnover using ^{15}N -lysine can explore the dynamic nature of endogenous protein metabolism. The incorporation of ^{15}N into urea is used to determine the oxidation rate of lysine. By priming both the lysine and urea pools, the infusion studies can be completed within two hours.

Lysine Protocol

Best Tracer: 1- ^{15}N -Lysine
Priming Bolus Dose: 5.0 $\mu\text{mol/kg}$ 1- ^{15}N -Lysine
Priming Bolus Dose: 1.5 $\mu\text{mol/kg}$ $^{15}\text{N}_2$ -Urea
Infusion Pump Speed: 0.174 cc/min
Infusion Rate: 3.0 $\mu\text{mol/kg/hr}$ 1- ^{15}N -Lysine only
Infusion Time: 120 min.
Sampling Times: 0, 80, 90, 100, 110, 120 min. (Plasma)
0, 60, 120 min. (Urine)
Diet Protocol: Fasted or Fed
References: Dietz et al, Metabolism 31:749-754, 1982
Shaw et al., Ann. Surg. 205:288-294, 1987

Leucine as a Tracer The amino acid leucine has been the most used tracer to determine whole body protein synthesis and breakdown rates. However, unlike the lysine model, the rate of leucine oxidation is quantified by measuring the production of $^{13}\text{CO}_2$. A modification with this model was developed by Wolfe which uses the enrichment of ketoisocaproate (KIC), the first metabolite of leucine oxidation, as the intracellular level of tracer. KIC appears in the plasma in sufficient concentration to be measured. It is generally believed that this is a good estimate of intracellular levels of tracer.

Leucine Protocol

Best Tracer: 1- ^{13}C -Leucine
Priming Bolus Dose: 4.64 $\mu\text{mol/kg}$ 1- ^{13}C -Leucine
Priming Bolus Dose: 0.10 mg/kg Sodium ^{13}C -Bicarbonate
Infusion Pump Speed: 0.174 cc/min
Infusion Rate: 4.2 $\mu\text{mol/kg/hr}$ 1- ^{13}C -Leucine only
Infusion Time: 140 min.
Sampling Times: 0, 90, 100, 110, 120, 130, 140 min. (Plasma and Breath)
Diet Protocol: Fasted or Fed
References: Motil et al., *Metabolism* 30,783-791, 1981
Motil et al., *Am. J. Physiol.* 240,E712-E721, 1981
Hoffer et al., *Br. J. Nutr.* 53,31-38, 1985
Matthews et al., *Metabolism* 31, 1105-1112, 1982

Phenylalanine as a Tracer This is a new model that has been verified by Thompson and co-workers (*Am. J. Physiol.* 256: E631-E639, 1989). The method obviates the need for measurement of expired CO_2 enrichment because it uses phenylalanine conversion to tyrosine as an estimate of phenylalanine oxidation. Phenylalanine can only be oxidized to carbon dioxide via conversion to tyrosine. By measuring the plasma isotope values of phenylalanine and tyrosine, one can determine whole body protein turnover. Priming the phenylalanine and tyrosine pools results in a 4 hour infusion compared to 12 hours, unprimed.

Phenylalanine Protocol

Best Tracer: L-[ring- $^2\text{H}_5$]-Phenylalanine
Priming Bolus Dose: 0.50 mg/kg L-[ring- $^2\text{H}_5$]-Phenylalanine
Priming Bolus Dose: 0.08 mg/kg L-[ring- $^2\text{H}_4$]-Tyrosine
Infusion Pump Speed: 0.174 cc/min
Infusion Rate: 0.50 mg/kg/hr
Infusion Time: 240 min.
Sampling Times: 0, 180, 190, 200, 210, 220, 230, 240 (Plasma)
Diet Protocol: Fasted or Fed
References: Thompson et al., *Am. J. Physiol.* 256, E631-E639, 1989
